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RESEARCH ARTICLE

PHYSIOLOGICAL QUALITY OF FIVE SOYBEAN CULTIVARS SUBMITTED TO WATER STREES

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ABSTRACT

During the period of seeds germination, many factors are determinant to the establishment of seedlings in field, being the water stress a negative effect. To create or adapt new cultivars to these conditions, plants breeding programs are fundamental. With this, the aim with this work was to verify the physiological quality of soybean seeds submitted to water stress. Were used seeds of five soybean cultivars (Savana, Conquista, Valiosa, BRS Celeste and Baliza) and five osmotical potentials (0, -0.2, -0.4, -0.6 and -0.8 MPa) diluted into water. To evaluate the physiological potential of these seeds, were realized the germination test with four replications of 50 seeds, distributed between three germitest paper moistened with PEG6000 solutions, described above. The evaluation was performed on the eighth day, with the first count realized determining the percentage of normal seedlings on the fifth day after planting and vigor evaluation of shoot and root length. The biochemical evaluation was performed by the expression of isoenzymes esterase (EST) and catalase (CAT). Based on the results was possible to observe that with the increase of water stress occurs reduction in the physiological quality, as well as reduction in the development of seedlings, independently of cultivar. The cultivar Savana is the most tolerant to water stress when compared to the others. There is variation in the expression of catalase and esterase in function of water stress levels in which seeds of soybean cultivars are exposed during the germination process.

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INTRODUCTION

With the increase of worldwide population there was a huge demand for food, and consequently one of the cultures which has been highlighted to supply this necessity of food is the soybean. The biggest part of utilization of soybean it is in the ration manufacturing for animals, oil production and recently has been increasing the consume in human feed. In the harvest 2013/2014, the worldwide production of soybean was approximately of 267,58 million of ton, with estimate of 285,05 for the harvest 2014/15, being the higher producer the United States, followed for Brazil (CONAB, 2015). Due to this big production, the soybean it is of fundamental importance to the Brazilian market being sufficient to attend the internal and external market requirements. In Brazil, seeds market started to increase in the beginning of 2007 when the country started to use in intensive form, many technological innovations, between them, the materials genetically modified what for soybean achieves more than 80% and the industrial treatment of soybean seeds which represents 40%. The utilization taxes of seeds (TUS) of soybean by the farmers is of 70%, what means seeds which passed by a quality control system. In light of, in Brazil there are more than 12 companies which commercialize 400.000 sacks of 40kg per year, representing a total of 34% of national trade (CONAB, 2014). Big productions like observed in soybean culture, is directly connected to many factors, being one of the most important, the quality of seeds that will be used. To express your maximum potential, the seed should be adapted to different regions in which have been cultivated. Even these same seeds, are subject to climatic problems, what year after year affects the agricultural in Brazil. In view of these problems, research institutions should be always a step ahead, mainly developing cultivars adapted to numerous problems existents, minimizing with this, the losses. By the fact of Brazil has a huge territorial extension, it is subject to some problems like, high or low temperature, salinity of soil, in some regions excess of rains and in others absence of rains etc.. these

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climatic conditions directly affects the agricultural production and in especial the germination and development of seeds. The water stress occurs when the supply of water is inefficient to the plant absorb in a way to replace the losses of water by the transpiration process (WU *et al*., 2007). Occurs by the absence of rains and irrigation in the area. Being the soybeana culture from places with mild temperatures and rain regularities, many breeding programs are working to create cultivars that overcome this absence of water available to the plant, bearing in mind that the soybean has being explored in areas driest, like the states of Mato Grosso, Tocantins and Goias, the biggest producers of this culture in Brazil. It is important to highlight that each cultivar of soybean present specific pattern of proteins that is synthetized, depending of the water stress conditions that the seeds are during the germinative process. In this way, the objective in this work, was to verify the physiological quality and pattern of enzymes expression catalase and esterase in soybean seeds from breeding programs under different concentrations of osmotical potential during the germination.

MATERIALS AND METHODS

This study was conducted at Central Laboratory of Seeds, Department of Agricultural of Universidade Federal de Lavras (UFLA), in Lavras-MG. Were used seeds of soybean cultivars savana, conquista, valiosa, BRS celeste and baliza, which were produced in experimental area of Universidade Federal de Lavras with soil classified like distrophic red latosol, with manual seeding. The multiplication of seeds was realized in a randomized block design with three replications. The experimental units had 4 lines of 5 meters each one, being considered only the 2 central lines like useful area. The thinning was realized keeping one population of 16 plants per linear meter. The manual harvesting was realized based on the phenological stages (R7 and R8) according with Fehr and Caviness (1977). The drying was realized in shadow, until seeds achieve 13% of water content. Were used seeds retained in sieves with circular opening between 5,55 mm and 6,35 mm for standardization of seeds seize for posterior realization of evaluations. Water content was determined by the oven method at 105°C for 24 hours, using two replicates of 50 seeds from each treatment (BRASIL, 2009). The results were expressed in percentage. In the germination test, for each treatment were used four replications of 50 seeds. Seeds were distributed between three germitest papers moistened with PEG6000 solution described according Table 1.

Table 1. Different concentrations of osmotical potential and conductivity, used to moisten the germitest paper to submit to water stress

Osmotical Potential (MPa)	Conductivity (μS dm^{-3})
0	0,08
-0,2	0,42
-0,4	2,41
-0,6	5,04
-0,8	9,53

Was used the amount of solution equivalent to 2,5 times the weight of dry paper. Following, the rolls were kept in germinator type B.O.D regulated to the constant temperature of 25ºC. The evaluation was realized at eighth day after the installation of the test by determining the percentage of normal seedlings (BRASIL, 2009). The first count was realized together to the germination test, by determining the percentage of normal seedlings at fifth day after the test installation. The results were expressed in medium percentage of normal seedlings of four replications. Were also determined the shoot and root length. For this, seeds from each treatment, in five replications of 15, were sowed equidistant from each other, according methodologies described to germination test. Was realized the measurement of hypocotyls and roots of seedlings classified like normals with an aid of a graduated ruler. The results were expressed in $cm.seedling^{-1}$. In the biochemical evaluation, samples of 25 seeds of each treatment were collected and macerated in presence of PVP (polyvinylpyrrolidone) and liquid nitrogen in small container and afterwards stored at -86ºC temperature. The seeds were collected 48 hours after the germination test installation.

For the enzymes extraction was added the extraction buffer (Tris HCl 0,2 M pH 8 + 0,1% of β-mercaptoethanol) in the proportion of 250μL for 100mg ofseeds powder. The material was homogenized in vortex and kept in refrigerator during 12 hours followed by the centrifugation at 14000 rpm for 30 minutes at 4ºC and them, applied in polyacrilamide gel. The electrophoretic run was realized in a discontinuous polyacrylamide gel system at 7,5% (separating gel) and 4,5% (concentrating gel) using Tris-glycine pH 8,9 as standard buffer in the gel electrode system. In each gel channel, was applied 50 µL of the sample supernatant and the running was performed at 150 V for 5 hours. At the end of running, the gels were revealed for the enzymes catalase (CAT- EC.1.11.1.6.) and esterase (EST- EC 3.1.1.1.), according the protocols established by Alfenas *et al*.(2006). The evaluation of the gels was realized on transilluminator, being considered the variation of intensity of bands. Was used the completely randomized experimental design in a factorial scheme of (5x5), being five soybean cultivars (Savana, Conquista, Valiosa, BRS Celeste and Baliza) and five different osmotical potential (0; -0,2; -0,4; -0,6 and -0,8 MPa). The datas, previously submitted to the normality tests and homocedasticity of variances, were submitted to analysis of variances and the averages were compared by the Scott-Knott test at 5% of probability. The statistical analyzes were realized with aid of SISVAR® statistical program (FERREIRA, 2011). The evaluation of the enzimatic patterns was made according to the intensity of the bands.

RESULTS AND DISCUSSION

Based on variance analyses (data not shown) was possible to observe significant differences between cultivars and between the osmotical potentials, as well as the interaction between the evaluated factors ($p < 0,05$). The medium water content of seeds in the moment of tests was of 14,1 with maximum variation of 1%. There was equilibrium hygroscopic, what means when seeds were placed in cold chamber was with 13% of moisture and when the germination test was initiate was with 14%, occurring equilibrium of moisture according the relative humidity and the temperature in cold chamber. In Table 2, the data related to root protrusion showed that in concentration of 0MPa the cultivar savanna obtained higher average, followed by the cultivar baliza, without significate difference the cultivars valiosa and BRS celeste. Inferior performance was observed to cultivar conquista. In relation to the concentration of -0,2MPa, there was a little change where savanna is still with the higher percentage followed by baliza. The other cultivars there were no significate difference.

Table 2. Percentage of root protusion at three days of five soybean cultivars, submitted to five levels of osmotical potential

Osmotical Potential (MPa)	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	44aA	22dA	27cA	25cA	33bA
-0,2	42aA	20cA	24cB	22cB	30bB
-0,4	37aB	16cB	20cC	17cB	27bB
-0,6	26aC	12bC	14bD	09cC	23aC
-0,8	12aD	08bD	11aE	06dC	12aD
CV (%)			40,97		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

In concentration of -0,4 MPa the profile was the same, the higher average was of cultivar savana, followed by baliza and again the cultivars valiosa, BRS celeste and conquista. In the concentration of -0,6Mpa, the cultivar BRS celeste presented the less percentage. In the lower concentration of -0,8MPa all the averages decreased. Analyzing each cultivar in different variations of osmotical potential was observed that all presented a decrease in the root protrusion and their averages also reduced when the osmotical potential decreases. Working with popcorn seeds, was observed that the reduction of osmotical potential causes reduction in the performance and development of popcorn seeds (MOTERLE *et al*., 2006), similar to the results founded in this work. When we analyze each concentration of osmotical potential in Table 3 for different cultivars, was observed that in concentration of 0MPa the cultivars savanna and baliza presented higher percentage of germination in the first count, and they didn't differ significantly being superiors in relation to the cultivars valiosa, conquista and BRS celeste that also, did not differ statistically.

Table 3. Percentage of normal seedlings in the first count of germination at five days of five soybean cultivars, submitted to five levels of osmotical potential

Osmotical Potential (MPa)	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	90aA	56bA	58bA	54bA	89aA
-0,2	79aB	43cB	46cA	42cA	70bB
-0,4	75aB	38cB	43cB	38cB	65bB
-0,6	63aC	23dB	39cB	34cC	53bC
-0,8	50aD	03cC	32bC	32bC	46aC
CV (%)			14,9		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

When analyzed the performance of each cultivar in different concentrations, all of them, without exception, decreases their averages when the concentration decreases. Working with bean seeds, it was conclude that the simulation of water stress in laboratory with the osmotic (mannitol, CaCl2, MgCl2 and NaCl) allows the confirmation of the stress effects in bean seedlings by the decrease of the parameters observed (COELHO *et al*., 2010). Equal to the decreases observed in this work. The comparison of concentrations to the cultivars in Table 4, showed that in the osmotic potential of 0MPa, the average of all cultivars showed practically to be the same.

Table 4. Percentage of germination of five soybean cultivars, submitted to five levels of osmotical potential

Osmotical Potential (MPa)	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	100aA	100aA	100aA	98aA	99aA
-0,2	97aB	89cB	92bB	88cB	91bB
-0,4	93aB	74cC	64cC	85bB	89bB
-0,6	91aB	68dD	60dC	70cC	77bC
-0,8	87aC	31dE	51cD	57bD	58bD
CV (%)			6,8		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

All the cultivars presented higher percentage of germination when submitted to higher concentrations of osmotic potential, and with the reduction of concentrations, were reducing the taxes of germination. Cultivar savanna presented lower variation of germination with the reduction of concentrations and the cultivar conquista presented the higher variation with the increase of concentrations when submitted to different osmotic potentials. Working with canola seeds, was verified that the reduction of the osmotic potential of substrate, promotes significate reduction in the germination of seeds and in the performance of canola seedlings. The level of osmotic potential of -1,0 MPa is critical to the germination of seeds and to development of seedlings (ÁVILA *et al*.,2007). Similar results to the higher concentration of -0,8MPa was found in this work. Data for shoot length are presented in Table 5, where was possible to observe that in lower osmotic potential of 0MPa, all cultivars except conquista, presented the same statistical averages.

Table 5. Shoots length of five soybean cultivars, submitted to five levels of osmotical potential

Osmotical Potential (MPa)	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	11,6aA	8,5bA	9,8aA	10,3aA	11,0aA
-0,2	8,7aB	8,4aA	7,2aB	8,7aB	6,3bB
-0,4	8,5aB	7,9aA	6,8bB	5,2cC	5,7cB
-0,6	7,0aC	5,2bB	1,6dC	4,1cD	2,4dC
-0,8	5,2aD	3,0bC	0,4dD	2,1cE	2,0cC
CV (%)			3,82		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

Campos (1990) working with rice seeds, observed that the length of seedlings was more reduced by the water stress than to the salt stress. The osmotic potential of -0,8 MPa of NaCl or Na_2So_4, can be considered critical to germination and growing of rice. In Table 6, the datas for root length showed the in the concentration of 0MPa the cultivar with lower average was valiosa, followed by the cultivars savanna, baliza, and with higher averages the cultivars BRS celeste and conquista.

Table 6. Roots length of five soybean cultivars, submitted to five levels of osmotical potential

Osmotical Potential (MPa)	Cultivars				
	Savana	Conquista	Valiosa	BRS Celeste	Baliza
0	7,9cA	13,8aA	5,7dA	14,5aA	9,5bA
-0,2	4,6cB	9,6aB	6,3bA	10,6aB	7,4bB
-0,4	1,2dC	2,1cC	5,1bA	7,2aC	5,9bC
-0,6	0,8cC	1,4bC	0,8cB	3,8aD	3,2aD
-0,8	0,6cD	0,4cD	0,5cB	2,1aE	1,9bE
CV (%)			4,76		

Means followed by the same lower case letter in line, and capital letter in column, do not differ at 5% probability by Scott Knott test.

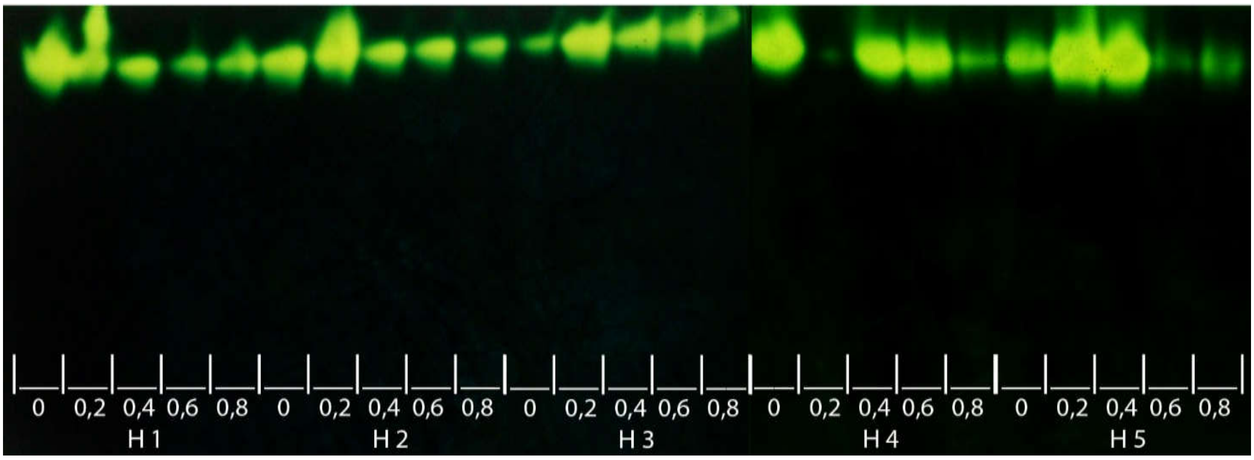

Figure 1. Pattern expression. of catalase enzyme in function of five cultivars (Savana, Conquista, Valiosa, BRS Celeste and Baliza), under five level of water stress

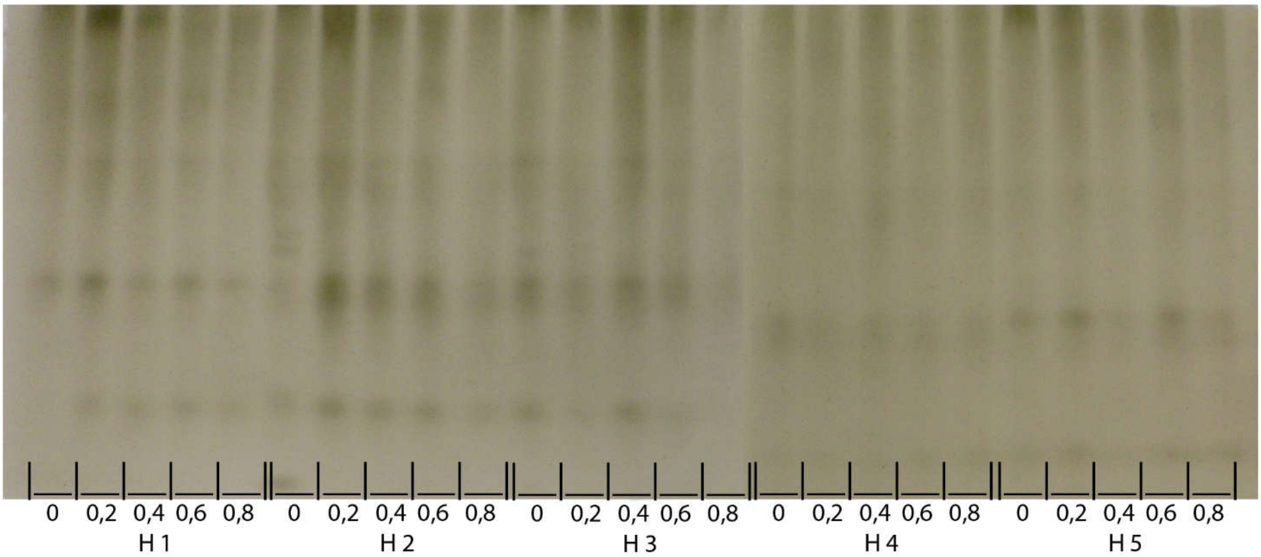

Figure 2. Pattern expression. of esterase enzyme in function of five cultivars (Savana, Conquista, Valiosa, BRS Celeste and Baliza), under five level of water stress

When we analyze the cultivars separated in each osmotic potential, is observed a significate decrease for all cultivars according the reduction in the osmotical potential were they were placed. In their work with garapa seeds and water stress, Spadeto (2012), observed that the length of seedlings reduced in linear form when the osmotic potentials decreases. These similar results were found here, in this work. In Figure 1, we have the pattern expression for the enzyme catalase in soybean seeds. Is possible to observe that with the decrease of osmotical potential, we have a reduction in the expression of this enzyme, independently of the cultivar analyzed. Enzymes catalase (CAT) and superoxide dismutase (SOD) are considered antioxidant enzymes, able to “clean” the reactive oxygen especies (ERO´s) from cells. Conditions of biotic stress which are imposed to the plants, leads to the overproduction of EROs what causes injuries in the cell structures affecting many times in an irreversible way (Barbosa *et al*., 2014). However, plants presents an efficient antioxidant system which is involved with the activity of a lot of enzymes, between them, catalase and esterase enzymes (ESTEVESandSUZUKI, 2008). Higher expression of CAT is related to the higher defense against the formation of H_2O_2celular (Barbosa *et al*., 2014). However, when the stress condition was increased, was possible to observe a decrease in the enzyme expression independently of the concentrations of PEG6000 used. This can be explained by the fact that in more severe conditions of stress, the cell decreases the production of CAT, what stay with the activity reduced, in order to decrease the cellular energetic spent. In Figure 2, we have the pattern of expression to the enzyme esterase in soybean seeds. Similar pattern to the observed to the enzyme catalase, was founded to the enzyme esterase, where is possible to observe that with the decreasing of osmotic potential we have the reduction of the expression of esterase enzyme for all the cultivars worked. The esterase (EST) is an enzyme associated to ester hydrolysis reactions and it is directly connected to the lipid metabolism and to the degenerative processes of cellular membranes (SANTOS *et al*., 2004). The reduction of expression of this enzyme with the increase of the stress can be justified by the double paper that this enzyme has, depending of the deterioration level resulting of some stress. It is important to highlight that this enzyme is accumulated before the deterioration process and, depending of the level of stress, can be the accumulation of this enzyme with the increase of deterioration, fact did not observe in this present work.

Conclusions

With increasing of water stress level, occurs the reduction in the physiological quality, as well as the reduction in the development of seedlings, independently of cultivar. The cultivar savana is more tolerant to water stress when compared to the others. There is variation in the enzymes catalase and esterase expression in function of stress levels where seeds of soybean cultivars are exposed during the germination process.

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